

A quantitative technique for the analysis of the gut contents of Collembola*

BY

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Although intermittently over the years there have been many observations of the food and feeding habits of Collembola (MACNAMARA, 1924; STREBEL, 1932; AGRELL, 1941; SCHALLER, 1950; DUNGER, 1956; POOLE, 1959; VON TÖRNIÉ, 1961, 1967) their nutrition is still little understood. Almost nothing is known of the physiology of digestion (although a start on this problem is now being made [ZINKLER, 1969]). But we are equally ignorant of the processes of food selection and intake and of the real nature of the diet of most species. CHRISTIANSEN (1964) has tentatively suggested a preliminary classification of diets and his review emphasises the lack of food specificity shown by most species, nearly all of which are apparently able to utilise a wide range of organic substances.

Recently, several authors (POOLE, 1959; HEALEY, 1965; KNIGHT and ANGEL, 1967; DE BERNARDI and PARISI, 1968; Gilmore and Raffensperger, 1970; BÖDVARSSON, 1970) have attempted to define the food of species more precisely by the analysis of gut contents of animals collected in the field. The weaknesses of this method are well known; it tends to over-emphasise the relative importance of durable, non-digestible materials, under-estimate the importance of quickly-digested materials and ignores liquid, transparent or minute substances. The results of food selection experiments are, however, generally highly-ambivalent (SCHALLER, 1950; DUNGER, 1956; KNIGHT and ANGEL, 1967; SINGH, 1969) and, at present, gut contents analysis is the technique most readily available for preliminary studies.

Generally, the Collembola are cleared in a clearing agent such as lactophenol. The gut contents are then microscopically viewed through the gut

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wall (BÖDVARSSON, 1970) or the gut contents are dissected out of the body and mounted alone either whole or as a squash (KNIGHT and ANGEL, 1967; GILMORE and RAFFENSPERGER, 1970). The materials present in the gut, mineral particles, fresh or dead plant material, fungal spores and hyphae, oligochaete setae, algae, pollen grains, arthropod cuticle, etc. are ranked for presence and absence, or frequency.

This paper describes a more quantitative technique for the analysis of gut contents, involving their dissection from the body of frozen animals, without clearing, and their filtration onto squared Millipore filters for quantitative analysis. Further details of some aspects of the technique and a more extensive series of results will be published shortly (ANDERSON and HEALEY in prep., McMILLAN in prep.).

Methods

Collembola are collected alive in their habitats by aspirator or paint brush and taken as soon as possible to the laboratory. Living animals are placed in small Pyrex crucibles, and asphyxiated with chloroform on cotton wool. Chips of carbon dioxide ice are added to the crucible: the Collembola are brought into contact with these and frozen. The frozen specimen is then placed on the stage of a dissecting microscope in a form-fitting groove in a pad of 'Plasticine' covered with 'Parafilm', an inert plastic sheeting (sold by Gallenkamp, Christopher Street, London E.C.2). Using two fine tungsten needles, drawn by electrolysis, the frozen body tissue is chipped away until the gut is exposed and can be removed intact. The gut wall is then softened by immersion for two to three minutes in a drop of 0.1 % lactic acid in distilled water in a small glass vial. The tissue of the gut wall is carefully teased away from the gut contents and removed from the vial. The gut contents are now carefully teased apart and the volume of the suspending fluid is brought up to two to three cc. by the addition of 0.1 % lactic acid. A stain may be introduced at this stage to assist the recognition of gut contents; a drop of 0.01 % methylene blue in distilled water has proved satisfactory. The vial is sealed and allowed to stand for some hours to allow uptake of stain.

The separated and suspended gut contents are now dispersed by vacuum filtration onto the surface of 'Millipore' filters, which are marked out in squares. A standard 'Millipore' microanalysis filter holder (Millipore (U.K.) Ltd., Catalogue No. XX 10,025,00) mounted on a vacuum flask is fitted with a moistened cellulose acetate filter with mean pore size $1.2 \mu \pm 0.3 \mu$ with a filtration area of 201 mm² or 12.6 mm² (Catalogue Nos RAWG 047, RAWG 013), depending on the amount of gut contents for analysis. The funnel of the filter holder is partially filled with distilled water, using a hypodermic to avoid air bubbles. Into this the contents of the vial, gut contents suspended in 0.1 % lactic acid, are carefully washed with several rinses of distilled water. Vacuum pressure at a constant 75 mm of mercury is then applied beneath the filter. During filtration the walls of the funnel are gently washed to prevent adhesion of particles. After filtration of the gut contents onto the filter is complete, the filtration pressure is maintained briefly to dry the filter partially. The filter is then removed and dried in an oven at low temperature (45-60° C) or left overnight at laboratory temperatures in a sterile petri dish lined with ordinary

filter paper. When dry the acetate filter is mounted between slide and coverslip in Canada Balsam, which makes the filter transparent; immersion oil may be used for this purpose in temporary mounts.

It is essential throughout the technique to ensure that all equipment is scrupulously clean and that solutions are pre-filtered through a filter with a pore size below $1.2\ \mu$.

Analysis of gut contents. The classification of gut contents that we have used is basically the same as that of the authors quoted above. With experience it would be possible to refine this classification. The following materials can be readily categorised:

- Particles of plant material $< 10\ \mu$
- Particles of plant material $10 \leftarrow \text{---} = 20\ \mu$
- Particles of plant material $> 20\ \mu$
- Fungal hyphae, diameter $< 2\ \mu$
- Fungal hyphae, diameter $> 2\ \mu$
- Fungal spores, aseptate
- Fungal spores, septate
- Pollen grains
- Collembolan remains (setae, cast skins, scales, etc.)
- Other arthropod remains
- Enchytraeid setae
- Mineral particles
- Undifferentiated fine material, $1.5\text{--}2\ \mu$.

The particles in each category on the filters are counted and the results expressed as percentages of the total number of particles present. [If the number of particles is great it may be necessary to count those on only part of the filter; this can be done by selecting squares at random]. The finest particles cannot be counted in this way. In this work we have estimated their abundance by a ranking procedure. The proportion of the filter covered by these particles in each field of view is estimated on a scale of 1-10, and this is averaged for the total surface of the filter. The filters are examined under oil immersion and phase contrast at a magnification of 1250 x.

Results

The technique has been developed for a study of feeding specificity of several species of *Onychiurus*. The preliminary results quoted here include data from some other species in various habitats. The data is given in Table 1. Five animals of each species from each habitat have been examined and the figures represent the mean percentages of the various identifiable materials found in their guts. The data could, if necessary, be subjected to a more rigorous statistical analysis.

The majority of the particles of higher plant material were amorphous, although in the two species of *Tomocerus* moss leaves were recognisable. The fungal spores and hyphae were mostly deuteromycetes, which include many

TABLE 1

The composition of the gut contents of seven species of Collembola analysed by the filtration method. Five individuals of each species were examined. Undifferentiated fine material is estimated by a ranking procedure described in the text

Species	Identifiable material : mean percentage of particles in gut contents														Undifferentiated fine material 1.5-2.5 μ
	Habitat	Mean number particles per gut $\pm$ S. E.	Plant material 10 μ ✓	Plant material 10-20 μ △	Plant material 20 μ △	Fungi				Pollen grains	Collembola remains	Enchytraeid setae	Mineral particles		
						Hyphae diam. 2 μ ✓	Hyphae diam. 2 μ △	Spores Asciolate	Spores Septate						
<i>Onychiurus furcifer</i> (Börner)	Nest of <i>Formica rufa</i> *	364 $\pm$ 71	77.5	2.7	1.1	1.2	8.5	2.4	0.6	0.7	0.9	1.4	3.0	2.5	
<i>Onychiurus armatus</i> (Tulb.) Gisin	Nest of <i>Formica rufa</i> *	214 $\pm$ 60	79.3	2.0	—	6.0	10.4	1.5	0.3	0.3	0.1	—	0.1	0.5	
<i>Onychiurus variabilis</i> Stach	Nest of <i>Formica rufa</i> *	619 $\pm$ 122	68.8	1.1	0.1	2.5	22.9	1.2	0.5	0.3	0.2	1.6	0.1	1.5	
<i>Tullbergia callipygos</i> Börner	Nest of <i>Formica rufa</i> *	278 $\pm$ 78	93.0	0.8	0.1	2.0	2.0	1.7	0.2	0.1	—	—	0.1	0.5	
<i>Lepidocyrtus lignorum</i> Fabricius	Compost (oak and grass)	1130 $\pm$ 223	26.7	0.5	—	0.5	0.2	72.0	—	0.1	—	—	—	1.0	
<i>Tomocerus minor</i> (Lubbock)	Beech litter *	1405 $\pm$ 60	63.5	4.0	1.4	1.7	25.6	2.6	0.4	0.5	0.2	—	—	0.3	
<i>Tomocerus flavescens</i> (Tullberg.)	Beech litter *	2145 $\pm$ 84	46.0	2.8	3.8	0.9	42.5	1.7	1.7	0.4	—	—	0.2	0.3	

* Blean Woods National Nature Reserve, Kent, June 1970.

common soil forms; some hyphae clearly contained cell contents. Undifferentiated fine material is a common component of collembolan gut contents. It seems to be organic in nature, but does not take up stain. In other work we have attempted to stain this material with bacterial stains, but without success; the possibility that it is bacteria, or is of bacterial origin, cannot however be ruled out.

This brief series of results demonstrates quantitatively the conclusions that have been reached by other workers with less rigorous techniques. That is, that the gut contents of soil and litter-dwelling Collembola consist of decomposing plant material mixed with smaller quantities of fungal hyphae, spores, pollen grains, mineral particles and quantities of finely divided, undifferentiated. It is difficult to avoid the conclusion that these species feed unselectively, and that their gut contents represent a random selection of the components of their environment.

The most interesting conclusion is that the four species of onychiurid Collembola living together in a well-defined micro-habitat, the nests of the wood ant *Formica rufa*, show no significant differences in their gut contents. General ecological theory suggests, in the principle of competitive exclusion, that species cannot live together if they share the same resources. This would lead one to expect differences of food or microhabitat between ecologically and morphologically similar species such as these. The same applies to the two species of *Tomocerus* from beech litter at Blean, though there are indications of small differences between these species. There remains, of course, the possibility of subtle microhabitat differences between these species (as suggested, in the case of *Tomocerus* spp. by the work of KNIGG, 1963) or alternatively differences of feeding that cannot be demonstrated by gut contents analysis (such as differences in gut enzyme complement, leading to selective absorption from the food, or coprophagy, etc.). These possibilities will be investigated in further work.

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RÉSUMÉ

Une technique servant à l'analyse du contenu intestinal chez les Collembolés est décrite. Des animaux prélevés sur le terrain sont congelés dans de la glace d'anhydride carbonique et l'ensemble des intestins est disséqué. La paroi intestinale est ramollie à l'aide d'acide lactique et enlevée. Le contenu intestinal est alors séparé, coloré au bleu de méthylène et passé sur des filtres en acetate de cellulose « Millipore ». Il est monté au baume du Canada qui le rend transpa-

rent. Les particules constituant le contenu intestinal sont comptés et la fréquence des différents composants estimée.

Une série de résultats préliminaires ne fait apparaître que peu ou pas de différence dans le contenu intestinal de quatre espèces de Collemboles de la famille des *Orcheselluridae* qui vivent ensemble dans un microhabitat bien défini. Quelques implications écologiques de ces résultats préliminaires sont mentionnées.

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